**Huntington’s Disease Gene Editing Design**

1. Purpose

Huntington’s disease is a fatal genetic disorder that causes the progressive degeneration of neurons in the brain and affects a person’s physical and mental abilities over time. Huntington's disease causes the breakdown of neurons specifically in a region of the brain called the Basal Ganglia. The Basal Ganglia are a group of subcortical nuclei primarily responsible for motor control, motor learning, behavior, executive functions and emotions. Over a 10-30 year period, a person will lose their ability to walk, talk, eat and care for themselves. Symptoms of Huntington’s disease include personality changes, depression, forgetfulness, impaired judgment, dystonia, chorea, impaired speech and gait, and it usually shows between the ages of 30 and 50. HD affects the whole brain, but some parts, such as the Basal Ganglia, are more vulnerable than others in the early stages of HD. It is crucial to cure Huntington’s disease because over 30,000 Americans have it, and their offspring have a 50% chance of getting this fatal disease too (regardless of gender and race).

1. Competing technologies

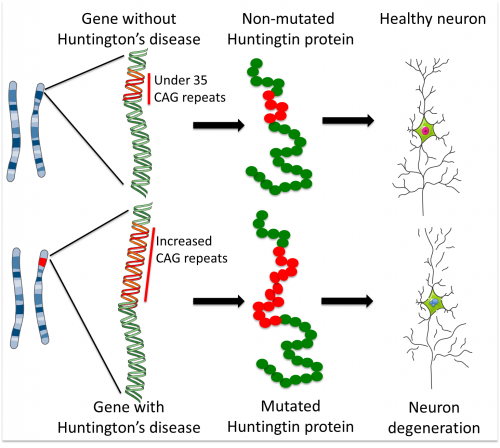
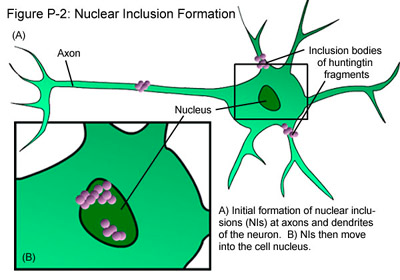
There is currently no known cure for Huntington’s disease. Its progress can’t be slowed down or reversed, which is the goal of many researchers and scientists. There are certain medications that can lessen the symptoms of HD, including tetrabenazine, benzodiazepines, antipsychotics and antidepressants, but these can often have negative side effects. The most promising treatments and possible cures to HD lie in the hands of researchers and scientists, who are conducting clinical trials and working with synthetic biology and genetic engineering to diminish the effects of HD before they occur. Many researchers are looking into gene therapies to cure HD.

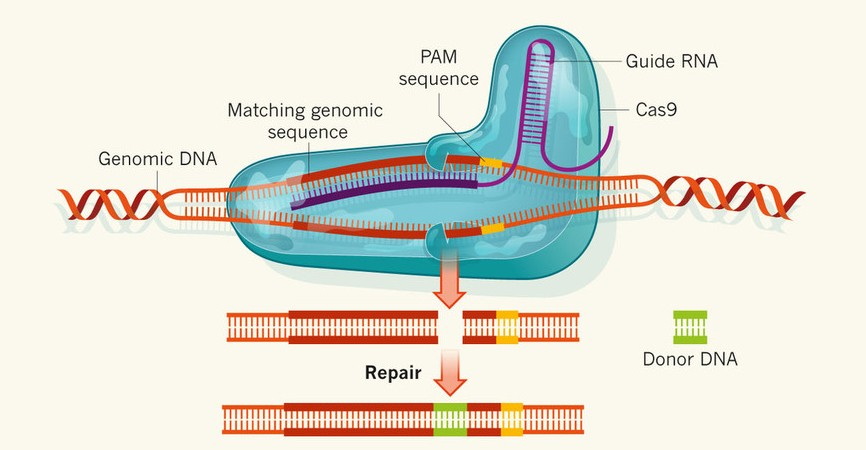
A group of scientists from Vancouver have developed a drug, ASO-HTT, that has been proven to halt and improve symptoms of HD in rats and monkeys. This drug is delivered through intrathecal injection, and can enter cells, stick to the mutant Huntingtin messenger RNA, and cause its degradation, stopping the Huntingtin protein from being made. Scientists are beginning clinical trials with this drug starting in August, and have already gathered 36 patients. This treatment does not use the most optimal delivery method, and patients would have to get the treatment every month. While it could improve the lives of HD patients, it is just a temporary treatment, and not a cure.

A company called Sangamo Biosciences is working to develop a treatment for HD using zinc finger nucleases (ZFN). Also working with the CHDI Foundation, Inc., Sangamo Biosciences have developed ZFNs that bind and cut the expanded CAG tract in the huntingtin gene. Their work proved beneficial in a mouse model when the part of the brain treated with ZFNs no longer produced the mutant huntingtin protein. The issue with this treatment is also the delivery of the drug, which does not give it access to all part of the brain. Therefore, the ZFNs only treated a portion of the mouse’s brain. With more research, Sangamo could develop a more effective delivery method of ZFNs to the brain.

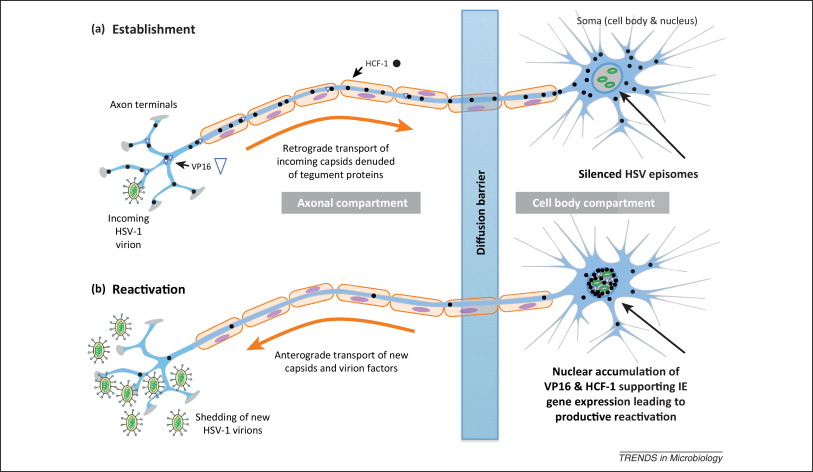
1. The design

Huntington’s disease is a genetic disorder that affects a gene in chromosome 4. Everyone has the huntingtin gene, but not everyone inherits the expansion, or mutation. Huntingtin gene is the gene responsible for the production of huntingtin protein, which plays a critical role in nerve cell function. This gene is expressed by a series of CAG trinucleotide repeats. Healthy people without HD have about 10-25 repeats of the genetic code CAG, whereas people with the expansion for HD have over 36. When someone has over 36 CAG repeats, their cells are instructed to create more huntingtin proteins than a healthy person. Since not all the proteins are needed, they are cut up into small, toxic pieces. These tiny excess pieces of huntingtin proteins float in synaptic space and penetrate neurons through their membrane pores, causing the breakdown of the neuron.



Our design will cut out the huntingtin gene mutation and replace it with a healthy one. We will do this by cutting out the mutated gene in the chromosome using CRISPR and Cas9. CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats, is an adapted prokaryotic immune system responsible for the resistance of foreign viral DNA such as those present in plasmids and phages. Cas9 is a nuclease protein that cuts the DNA sequence where CRISPR RNA guides it to. This system of acquired immunity allows CRISPR and Cas9 to recognize and cut the exogenous gene element so that it cannot infect the cell.

By synthesizing this naturally occurring gene editing device, we can program CRISPR RNA to guide Cas9 to a specific gene sequence when it encounters neural DNA and replace the huntingtin gene expansion with donor DNA that has the appropriate amount of CAG repeats. We will deliver programmed CRISPR Cas9 into neurons through a viral vector. rHSV-1 is a recombinant version of the Herpes Simplex Virus (HSV-1), with many dangerous viral genes removed, to make it safer for CNS use. rHSV-1 vectors are ideal for CNS integration because they can pass the blood brain barrier, they show minimal cytotoxicity and inflammatory properties, have natural neurotropism via axoplasmic transport, and have high transgene expression.



The rHSV-1 virus will inject CRISPR Cas9 into neurons, or “infect them”. Once in the neuron, Cas9 will latch onto the neural DNA and the guide RNA will specify which gene sequence to cleave. Cas9 will cut out the HD gene, and replace it with the Donor DNA. This will occur throughout all of the neurons this virus infects, curing the disease before it becomes effective.

1. Expected results

With this design, we expect neurons to have the appropriate amount of CAG trinucleotide repeats. Since we will be replacing the mutated gene with a healthy, normal gene, we will expect the patient not to develop HD, therefore sparing their brain of degradation.

Truth Table:

|  |  |  |
| --- | --- | --- |
| CRISPR Cas9 DNA | Huntingtin gene expansion | Huntington’s Disease |
| 1 | 0 | 0 |
| 0 | 1 | 1 |

The truth table signifies that (for people with HD) when CRISPR is present, they will not have HD. If CRISPR is not present, the patient will still have HD and the huntingtin gene expansion.

1. Advantages

The advantages for our treatment design are vast. To cure Huntington's Disease would be to save the lives of tens of thousands of people, save them from the torture of slowly losing control of their mind and body, and to save their family from mourning and loss. This design could lead to the development of many other gene therapy treatments to cure other serious genetic disorders.

Our design not only surpasses medications to lessen symptoms, it gets rid of Huntington’s disease as a whole. Most existing technologies are temporary treatments rather than cures, as stated in Section II. However, the ZFN treatment developed by Sangamo Biosciences is similar to CRISPR because both are designed to edit the huntingtin gene. With extensive research and experimentation, their treatment proved beneficial on mice, but the drug does not reach all parts of the brain. Our design consists of an optimal delivery system that reaches all nerve cells the brain. This design is worth funding because it has the potential to cure Huntington’s disease and lead to similar treatment for other neurological disorders.

1. Potential problems

Risks and danger always accompany technological designs. When editing the DNA of cells, we face many potential problems. Viruses can infect more than one type of cell, for example cells in the circulatory system may be infected when the virus is injected rather than only cells in the nervous system. Scientists and researchers who are working on this project and are exposed to viruses could get infected by the rHSV-1 virus even with protection in the sterile environment. Due to the danger this poses on employees in the lab, we will include safety equipment such as gloves, masks, goggles and proper sanitation. There may also be a possibility that the new genes could be overexpressed. For example, if the Cas9 protein cuts too much of the Huntington’s disease sequence to less than 10 repeats of CAG, it may be harmful to the cell. On another hand, this CRISPR design may evolve and negatively affect the neurons by hypothetically not cutting the huntingtin gene where it should, or the virus could evolve and infect the neurons with viral DNA instead of CRISPR.

1. Testing

We will begin our research by doing tests on lab mice with Huntington’s disease. We will use a viral vector appropriate for mice to inject CRISPR Cas9 into their neurons. Doing so will help us identify any side effects the viral vector may cause or if it even effectively carries and delivers CRISPR at all. Testing can also identify any room for improvement that was not thought of originally. For example, if the virus causes headaches, we can alter it to not only prevent that, but make sure it does not harm the brain and nervous system in any other way. We will also specifically test whether CRISPR RNA will be able to identify the difference between a few repeats of CAG that may occur in multiple chromosomes and over 30 repeats of CAG that occurs in only chromosome 4 by trial and error of programming CRISPR. After success in tests with lab mice, we can begin to test on monkeys, and then even humans. A clinical trial would be optimal for discovering the effects of our design.

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